

Experimental study of microbial P limitation in the eastern Mediterranean

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Abstract

In this study we experimentally tested the hypothesis that phosphorus was the primary nutrient limiting phytoplankton and bacterial growth in the eastern Mediterranean Sea, and examined the spatial variability in P limitation during winter. Complementary measurements were employed using water sampled during January 1995 from nine pelagic stations east of the Straits of Sicily. Ambient concentrations of inorganic P (P_i) in the upper 50 m of the water column in seven of the stations were 20–40 nM. The upper limit of bioavailable P ranged from 6 to 18 nM, suggesting severe P shortage. Orthophosphate turnover time ranged from 2 to 7 h in those P_i -depleted waters. In nutrient-enrichment bioassays using subsurface water from the Ionian and Levantine basins, P addition caused significant increases in bacterial activity, bacterial numbers, and chlorophyll *a* relative to unenriched controls. The addition of $\text{NH}_4^+ + \text{Fe} + \text{EDTA}$ did not have these effects. In a similar bioassay using Cretan water, microbial growth was obtained even in the unenriched controls, suggesting that other factors (e.g. grazing, light) were influential. Higher ambient P_i concentrations were encountered in the Cretan Sea (90 nM) and in the core of the Rhodes gyre (210 nM), where our sampling coincided with a convective mixing event. In those stations, P sufficiency was indicated. We concluded that in the pelagic waters of the eastern Mediterranean in winter, P was the primary limiting nutrient when other factors (such as light or grazing) did not control microbial biomass or activity. In ultra-oligotrophic waters, a delicate and dynamic balance differentiates between times when the microbial populations are nutrient limited and times when growth becomes limited by other factors. We caution that the interpretation of data obtained using conventional methods that were developed and tested in more enriched systems may not be valid in ultra-oligotrophic systems.

In most of the world's oceans, growth and production of planktonic microbial (bacteria and algae) populations were traditionally considered to be limited by the availability of nitrogen. This dogma was challenged by Hecky and Kilham (1988), leading to its reevaluation. Recently, it was demonstrated that algal production in the nitrogen-rich, biomass-poor equatorial Pacific was limited by iron availability (Martin et al. 1994; Kolber et al. 1994). There is growing evidence that phosphorus may be the main limiting nutrient in some coastal systems (Thingstad et al. 1993) and in oligotrophic oceans, particularly in the Sargasso Sea (Cotner et al. 1997). Karl et al. (1995) claimed that the subtropical

North Pacific has recently shifted from N limitation to P limitation.

The idea that the Mediterranean Sea may be P limited is not new (Berland et al. 1980). Krom et al. (1991) claimed that, indeed, P was the limiting nutrient to planktonic production in the eastern Mediterranean Sea based on the disappearance of orthophosphate before nitrate from surface water and on anomalously high ratios of dissolved inorganic N:P in the deep water. However, their claim has not been verified experimentally. For the western Mediterranean, experimental evidence is accumulating that at least during part of the year P is the limiting nutrient (Zweifel et al. 1993; Thingstad and Rassoulzadegan 1995; Berdalet et al. 1996; Vaultot et al. 1996).

The eastern Mediterranean is ultra-oligotrophic (Yacobi et al. 1995), with summer phosphate concentrations in the upper mixed layer usually below the detection limit of 10 nM, nitrate below 0.6 μM , chlorophyll *a* below 250 ng liter⁻¹ (Krom et al. 1991), primary production of $\sim 45 \text{ mg C m}^{-2} \text{ d}^{-1}$, and heterotrophic bacterial production about half that amount (Robarts et al. 1996). Phytoplankton populations are dominated numerically by picoplanktonic forms, with prochlorophytes and cyanobacteria (*Synechococcus* sp.) as primary components (Li et al. 1993). Bacterial biomass constitutes $\sim 50\%$ of algal biomass (Robarts et al. 1996). The oligotrophic conditions tend to be more extreme with increasing distance from the Atlantic Ocean, the major source

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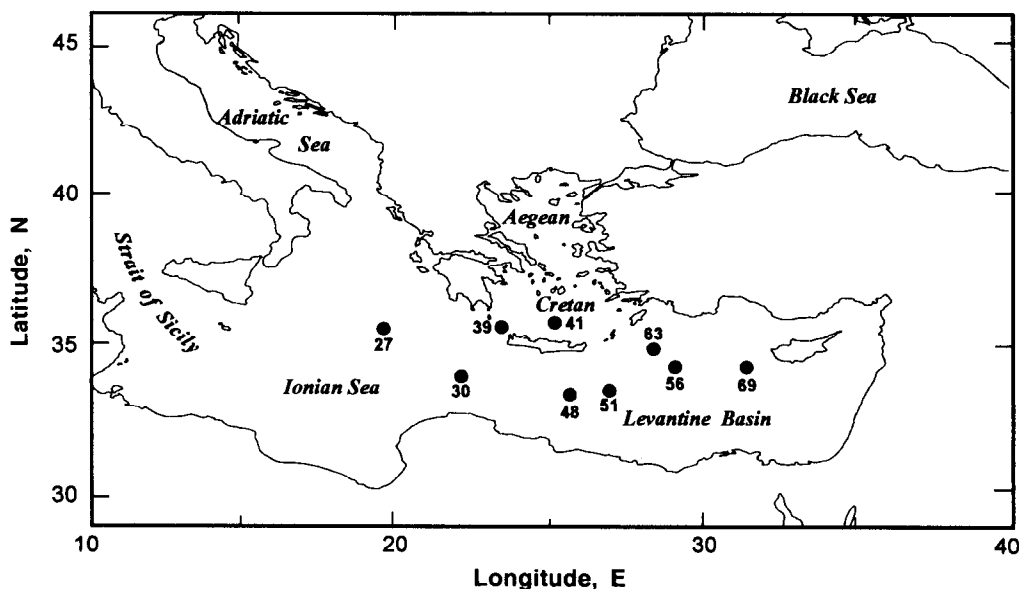


Fig. 1. Map of the eastern Mediterranean Sea showing stations sampled during the *Meteor* M31/1 cruise, 17 January–5 February 1995.

of nutrients, and with increasing distance from the land-enclosed Aegean and Adriatic Seas.

In this study we experimentally test the hypothesis that P is the primary nutrient limiting the growth of current microbial populations in the eastern Mediterranean and examine the spatial variability in the extent of P limitation. In oligotrophic oceans, bacterial biomass is often as high as phytoplankton biomass (Cho and Azam 1990; Li et al. 1992), and thus our investigations have focused on both phytoplankton and bacteria. With no single method yet available to definitively demonstrate P limitation, we used a suite of complementary measurements, all related to the P dynamics in water. Jointly, these data enabled us to conclude that, in winter, P was potentially the limiting nutrient to microbial biomass and production when other factors (light, grazing) were not dominating.

Table 1. Chlorophyll *a* concentration, bacterial numbers, and their ratio throughout the eastern Mediterranean upper mixed layer during January 1995. Station locations are shown in Fig. 1.

Region	Sta.	Depth (m)	Chl (ng liter ⁻¹)	Bacteria (10 ⁶ liter ⁻¹)	Chl : bacteria
Ionian	27	50	120	3.05	39.3
Ionian	30	50	155	2.95	52.5
Aegean outflow	39	50	133	1.56	85.3
Cretan	41	50	135	1.57	86.0
Levantine	48	50	188	2.75	68.4
Marsa Metreuh	51	50	118	3.35	35.2
Levantine	56	40	360	3.58	100.6
Rhodes gyre	63	80	260	2.87	90.6
Levantine	69	20	180	2.96	60.8
Levantine	69	75	245	3.91	62.7
Mean			189.4	2.9	68.1
(SD)			(77.8)	(0.8)	(22.1)

Methods

Study site and sampling—The study was carried out aboard the RV *Meteor* during cruise M31/1 to the Mediterranean, 19 January–5 February 1995. Water samples were collected from nine stations, which were selected to represent the main basins or physical features of the Mediterranean Sea east of the Straits of Sicily (Fig. 1). All water samples were collected using a rosette system equipped with 15 liter Go-Flo bottles (General Oceanics) mounted on a CTD with an in situ SeaTech fluorometer. Samples were collected from the depth of maximum fluorescence (usually ~50 m), or from 50 m when no such maximum was observed (Table 1). At Sta. 69 in the Levantine Basin additional samples were collected to give a depth profile throughout the upper mixed layer. The following parameters were determined in duplicate or triplicate on each of the water samples: Chl *a* nitrate, and phosphate concentrations, bacterial numbers and activity (³H]thymidine method), alkaline phosphatase activity, and size-fractionated ³²P uptake. On several occasions the upper limit to bioavailable P was also determined.

Nutrient and biomass determinations—Samples for nutrient concentrations were collected and frozen (–20°C) in acid-washed vials that were rinsed twice with sample water before filling. Upon return to the laboratory, nitrate and orthophosphate concentrations were determined by means of a Technicon autoanalyzer (Krom et al. 1991). Chl *a* was determined fluorometrically (Holm-Hansen et al. 1965) from cold acetone extracts of 250 ml samples filtered through GF/F filters immediately after sampling. The filters were kept frozen (–20°C) until analyzed, within a week after the end of the cruise. Bacterial samples were preserved with Lugol's solution + formaldehyde (Sherr et al. 1989) and counted with an epifluorescent microscope after staining with DAPI.

Activity parameters—Bacterial activity was followed using the [^3H]thymidine method as modified for oligotrophic waters by Robarts and Zohary (1993), with the exception that the DNA-extraction step (phenol/chloroform) was omitted. As a result, the data provide a sensitive measure of bacterial activity via an estimate of the rate of thymidine incorporation into macromolecules. At the substrate concentrations used, the method gives a clean signal of heterotrophic bacterial activity that is not compounded by potential phytoplankton activity (Robarts and Zohary 1993).

Alkaline phosphatase activity (APA) was measured to evaluate the relative P deficiency of the microbial assemblage (Pettersson 1980). Through this enzymatic process, organic P is converted to orthophosphate—a strategy used by algae and bacteria to gain P when inorganic P (P_i) is in limiting concentrations. Duplicate 200-ml samples were filtered through 0.2- μm filters, which were immediately frozen (-20°C) and then transported to the laboratory on dry ice. Upon return to the laboratory, APA was measured spectrophotometrically with 4-methylumbelliferyl phosphate (MUP) as the substrate (Pettersson 1980). This freezing procedure was tested and proven not to alter the APA results (O. Hadas unpubl. data). Enzymatic activity was expressed as pmol MU liter $^{-1}$ d $^{-1}$ and normalized to Chl *a*, giving APA as nmol MU ($\mu\text{g Chl}$) $^{-1}$ d $^{-1}$.

^{32}P uptake and turnover times— ^{32}P -uptake experiments were carried out to measure the rate at which P_i is removed from the water by the microbial community: as P_i becomes more plentiful, its turnover time becomes longer. Uptake measurements began within 1 h of water collection, in the onboard radioisotope laboratory, according to methods outlined by Bentzen and Taylor (1991). Carrier-free $^{32}\text{PO}_4^{3-}$ -P (Amersham, PBS11), diluted $\times 50$ with distilled water, was added to 100-ml samples to give a total activity of 10^5 CPM ml $^{-1}$. Triplicate 1-ml aliquots of the incubation liquid were withdrawn and placed in 3 ml of scintillation fluor (Ultimagold, Packard Instruments) to verify the amount of ^{32}P added to each sample. At timed intervals, 5-ml aliquots were drawn and passed through 0.2- μm pore-size Sartorius cellulose nitrate filters. To minimize background counts, filters were pre-soaked in a cold orthophosphate solution (0.2 mM). After filtration, the filters were rinsed twice with 3 ml of freshly prepared filter-sterilized seawater. Formalin-killed controls were run in the same manner to determine background ^{32}P -uptake levels, which were then subtracted. The filters with particulate ^{32}P were dissolved for >2 h in minivials containing 0.75 ml ethyl acetate, and then 3.5 ml of Ultimagold scintillation fluor was added. Radioactivity was measured with an onboard liquid scintillation spectrometer. Turnover times were calculated from plots relating the natural log of the percentage of ^{32}P remaining in the filtrate vs. the time of the incubation (Bentzen and Taylor 1991).

Size-fractionation of ^{32}P uptake was used to ascertain which portion of the microbial population was responsible for most of the P uptake. A preliminary test carried out in triplicate using surface pelagic Levantine water revealed that 83% of Chl *a* was retained on 1- μm pore-size filters, while practically all free bacteria passed through. Thus, a 1- μm cutoff was a convenient way of separating predominantly

algal from predominantly bacterial effects. A 10-ml aliquot of the radiolabeled water was withdrawn and filtered through a plexiglass dual-filtering tower using a 1.0- μm pore-size filter (Nuclepore) on the top frit and a 0.1- μm pore-size filter (Sartorius) on the bottom. Filters were treated as above and were counted in the scintillation counter.

Concentration of biologically available P—The upper boundary of biologically available P was determined on various occasions from ^{32}P uptake and Michaelis–Menten equations as the parameter $K_s + P_n$ according to Rigler's radio-bioassay method as described by Bentzen and Taylor (1991).

Nutrient-enrichment bioassays—Enrichment bioassays were conducted in 12 2-liter presterilized Nalgene polycarbonate bottles filled with prescreened (100- μm mesh) water from 50-m depth. Three such experiments were conducted with water from Sta. 27 in the Ionian Sea, Sta. 41 in the Cretan Sea, and Sta. 56 in the Levantine Basin. Nutrients were added in four triplicate treatments in a factorial design: addition of orthophosphate (P); addition of NH_4^+ , Fe, and EDTA (designated "other nutrients," or ON); addition of all of the above (ON + P); and no addition (control). Nitrogen was added as NH_4Cl at a final concentration of 2 μM , $\text{FeSO}_4 \cdot 17\text{H}_2\text{O}$ at 0.1 nM, EDTA at 0.1 μM , and KH_2PO_4 at 0.1 μM . N and P were added at a molar ratio of 20:1, characteristic of the Levantine Basin deep water (Krom et al. 1991). The bottles were incubated for 3 d at room temperature (19°C) under continuous light ($\sim 30 \mu\text{Einst m}^{-2} \text{s}^{-1}$). They were periodically hand-shaken and their order altered randomly. Water samples were taken daily for measurement of bacterial activity, and at the start and end of the experiment for determination of the Chl *a* concentration and bacterial numbers, as described above.

To test the significance of the effect of each treatment combination, a repeated-measures two-way ANOVA was used for the bacterial activity parameter. For the biomass parameters, Chl *a*, and bacterial numbers, the rate of biomass change, r , was computed using $r = [\ln(\text{Start value}/\text{End value})]/\text{Incubation time}$. A two-way ANOVA was run on these r values.

Results

Cross-basin variability—Near-surface water temperature during this winter cruise ranged from 14 to 16°C . An upper mixed layer, usually extending to 100–150-m depth existed at most stations, with a few exceptions. At the Cretan Sea, a prominent front was observed between Sta. 39 and 41. At Sta. 41, a well-mixed upper layer extended to 200-m depth, with no distinct temperature, salinity or fluorescence maximum. The water column at the Aegean outflow (Sta. 39; 800-m depth) was isohaline with a gradual thermocline spread over the upper 400 m. A convective chimney was found in the Rhodes gyre (Sta. 63), with a uniform-density water column throughout its entire 2,900-m depth (Malanotte-Rizzoli et al. 1996).

Nutrient concentrations in the upper mixed layer of most stations (Table 2) were typically 20–40 nM PO_4^{3-} with variable NO_3^- (range of 0.13–1.5 μM). Significantly higher con-

Table 2. Nutrient concentrations and P dynamics-related parameters for the eastern Mediterranean upper mixed layer, January 1996. Station locations are shown in Fig. 1 (APA, alkaline phosphatase activity; TdR, [³H]thymidine method; T_i , P turnover times; $K_s + P_m$, upper limit of bioavailable P [see text]).

Region	Sta.	Depth (m)	PO ₄ ³⁻ (nM)	NO ₃ ⁻ (μM)	APA, (pmol MU liter ⁻¹ d ⁻¹)	Spec. APA (nmol MU μg Chl ⁻¹ d ⁻¹)	TdR (pmol liter ⁻¹ h ⁻¹)	Spec. TdR (10 ⁻²¹ mol cell ⁻¹ h ⁻¹)	T_i (h)	% ³² P in <1.0-μm fraction	$K_s + P_m$ (nM)
Ionian	27	50	20	0.3	~0	~0	0.12	0.39	2.9	—*	—
Ionian	30	50	40	0.1	315	2.0	1.53	5.19	6.7	78.6	—
Aegean outflow	39	50	35	1.5	1,283	9.7	0.51	3.27	828	63.3	17.7
Cretan	41	50	90	3.6	338	2.5	0.43	2.74	150	—	10.6
Levantine	48	50	40	0.8	1,598	8.5	0.39	1.42	5.1	61.2	—
Marsa Metreuh	51	50	40	0.3	5,805	49.2	0.87	2.60	5.6	60.5	—
Levantine	56	40	20	1.0	1,350	3.8	1.68	4.69	2	84.3	—
Rhodes gyre	63	80	210	4.1	1,778	6.8	0.67	2.33	3,711	39.7	†
Levantine	69	20	25	0.3	315	1.8	0.45	1.52	3.6	77.6	8
Levantine	69	75	25	0.8	473	1.9	0.57	1.46	6.1	77.2	6.2
Mean			58.5	1.2	1,326	9.6	0.7	2.6		67.8	8.3
(SD)			(57.0)	(1.3)	(1,695)	(15.2)	(0.5)	(1.5)		(14.5)	(2.2)

* Mean was not measured.

† Could not calculate owing to long turnover time.

concentrations were recorded at the core of the Rhodes gyre (~200 nM PO₄³⁻ and 4–5 μM NO₃⁻ throughout the entire water column) and at the Cretan front (90 nM PO₄³⁻ and 3.6 μM NO₃⁻).

Chl *a* concentrations at the depth of maximum fluorescence (40–80 m) ranged from 118 to 360 ng liter⁻¹, with an overall mean of 190 ng liter⁻¹ (Table 1). The concentrations were distinctly higher at the core and vicinity of the Rhodes gyre (Sta. 56 and 63) and in the eastern Levantine (Sta. 69) than at the more western stations.

Bacterial numbers in 7 out of 9 stations fell within the relatively narrow range of 2.8–3.9 × 10⁸ cells liter⁻¹, but were considerably lower (1.6 × 10⁸ cells liter⁻¹) in the Aegean outflow and the Cretan Sea. The ratio Chl/bacterial number (in ng Chl per 10⁸ cells), which gives a means of comparing the relative biomass of phytoplankton and bacteria at the different sites, ranged from 35.2 to 100.6, with an overall mean of 68.1 ± 22.1 (SD).

Bacterial incorporation rates varied by about an order of magnitude across the eastern Mediterranean, ranging from 0.12 pmol liter⁻¹ h⁻¹ at Sta. 27 in the Ionian Sea to maxima of 1.68 pmol liter⁻¹ h⁻¹ at Sta. 56 and 1.53 pmol liter⁻¹ h⁻¹ at Sta. 30 (Table 2). As expressed (per bacterial cell), the spatial pattern resembled that of the activity per unit volume of water. In comparison with rates of thymidine incorporation measured throughout the Levantine basin in October–November 1993 (Robarts et al. 1996), the rates recorded in this study tended to fall within the upper range, but this was expected as the DNA extraction step was omitted, and the study was conducted during winter mixing when nutrients concentrations were generally higher.

Alkaline phosphatase activity (APA; Table 2) ranged from below detection at Sta. 27 (Ionian), where bacterial activity was also lowest, to 5,800 pmol MU liter⁻¹ d⁻¹ at Sta. 51 (western Levantine), where the specific activity per unit Chl *a* (spec. APA) was highest. APA values did not clearly correspond with any of the other parameters measured (ambient

PO₄³⁻, Chl *a* concentrations, bacterial numbers, bacterial activity, or P turnover times). Unfortunately, the substrate for APA, dissolved organic P (DOP), was not determined.

Phosphorus turnover times (T_i) varied more than three orders of magnitude, being fastest in the Levantine Basin (Sta. 56) at 2 h and slowest in the Rhodes Gyre core (Sta. 63) at 3700 h (Table 2). The T_i data could not be interpreted using cutoff values derived from studies in freshwater systems, where T_i of >30 min typically indicate P sufficiency (Lean and Nalewajko 1979). In the oligotrophic oceans the extreme scarcity of organisms leads to much longer turnover times. Suttle et al. (1990) suggested that values of 2–5 h indicated P deficiency in the Sargasso Sea. Based on the supporting data we have, we propose that values up to 7 h indicate P deficiency in the eastern Mediterranean. Using this cutoff, our data suggest that P-limited conditions predominated in the eastern Mediterranean, with the exception of P-enriched sites.

³²P turnover times were often high where APA was low and vice-versa. This demonstrates that the utilization of different sources of P (i.e. dissolved organic vs. dissolved inorganic P) may operate at different relative rates, depending on environmental conditions.

With the exception of the P_i -enriched Rhodes gyre core, ³²P uptake was dominated (>60%) by the bacterial size fraction (0.1–1 μm). In the Rhodes gyre, the situation was reversed and the algal (>1 μm) fraction dominated ³²P uptake.

Our derived upper boundary of bioavailable P, $K_s + P_m$, were consistently lower than the measured PO₄³⁻ values, suggesting that not all the P measured as PO₄³⁻ in our chemical analyses was biologically available. We expected the P turnover time, T_i , to become increasingly shorter as PO₄³⁻ concentrations declined. In fact, in 6 of 7 stations where PO₄³⁻ did not exceed 40 nM (Table 2), T_i clustered at values <7 h, while at 2 of 3 stations with elevated PO₄³⁻, T_i exceeded 100 h. However, in one case (Sta. 39) T_i was long despite low PO₄³⁻, and in one case (Sta. 56) T_i was short

Levantine Basin, Sta. 69

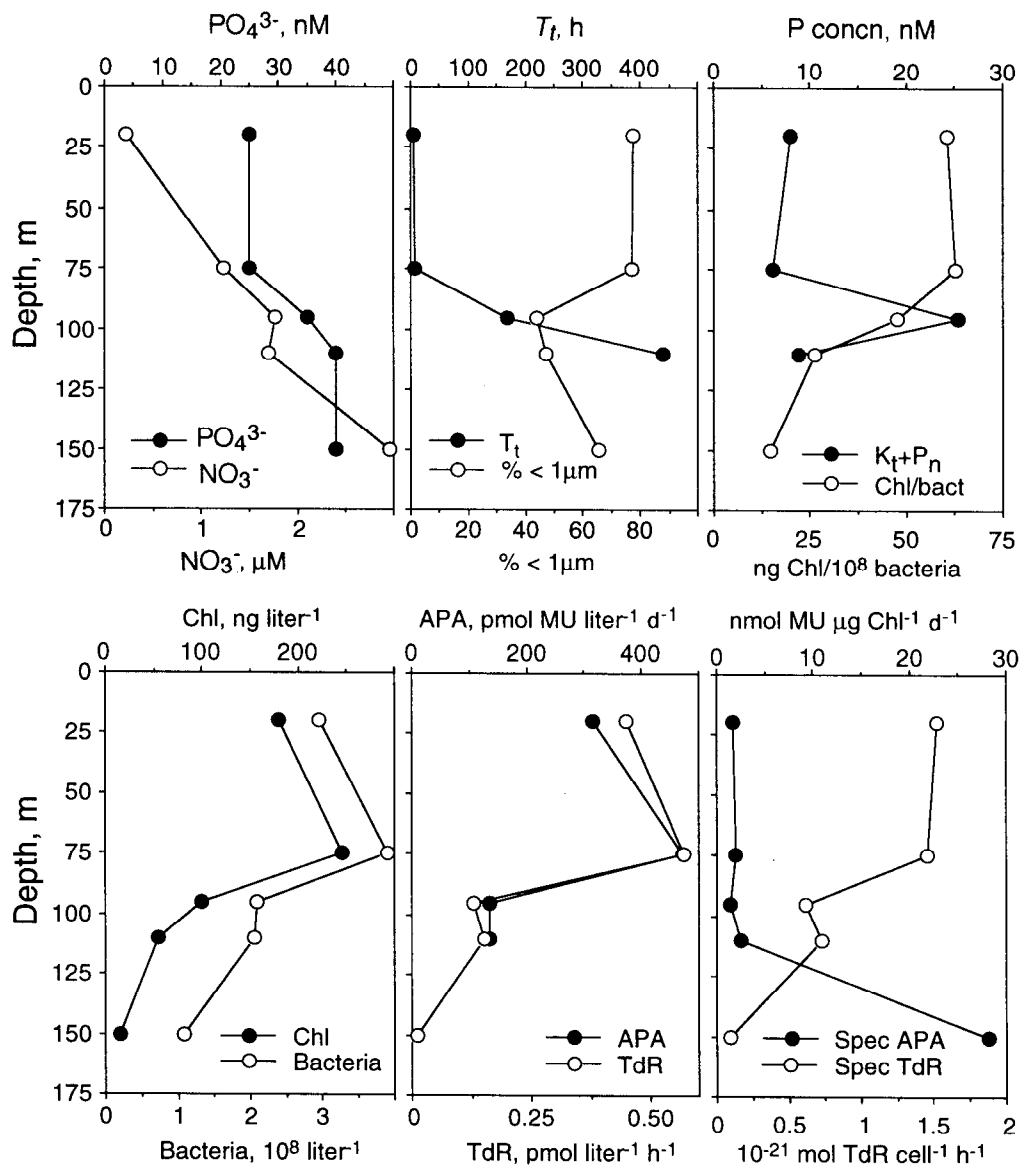


Fig. 2. Depth distribution of nutrients (PO_4^{3-} , NO_3^-) and of P dynamics parameters at Sta. 69 (Levantine Basin), 31 January 1995. T_t , phosphorus turnover time computed from ^{32}P uptake; % < 1 μm , proportion of ^{32}P uptake due to the < 1- μm (mostly bacteria) fraction; $K_t + P_n$, the upper limit of bioavailable P; Chl, chlorophyll *a* concentration; Chl:bact, the ratio of Chl *a* to bacterial number (ng Chl/10⁸ bacteria); APA, alkaline phosphatase activity; Spec. APA, APA expressed per unit Chl *a*; TdR, [^3H]thymidine incorporation rates; Spec. TdR, TdR values expressed per bacterial cell.

despite high PO_4^{3-} , suggesting other complicating factors. Similarly, PO_4^{3-} was not a good predictor of APA, as both high and low APA values corresponded to similarly low PO_4^{3-} concentrations.

Depth distribution—At Sta. 69, an upper mixed layer of ~150 m existed, with a temperature of ~15.5°C and salinity of 39.0 psu. Orthophosphate concentrations, 25–40 nM in the upper 110 m (Fig. 2), were similar to those elsewhere in the eastern Mediterranean. Nitrate concentrations increased

with depth, from 0.23 μM at 20 m to 3 μM at 150 m. With the exception of the Rhodes gyre and the Cretan front, this depth profile covered the entire range of nutrient concentrations in our spatial distribution survey, demonstrating a shift from P-deficient to P-sufficient conditions with increasing depth.

A distinct biomass peak of both phytoplankton (as Chl *a*) and bacteria was observed at 75 m, beneath which both had declined dramatically by 150 m (Fig. 2). The Chl:bacteria ratio also declined beneath 75 m, suggesting that with in-

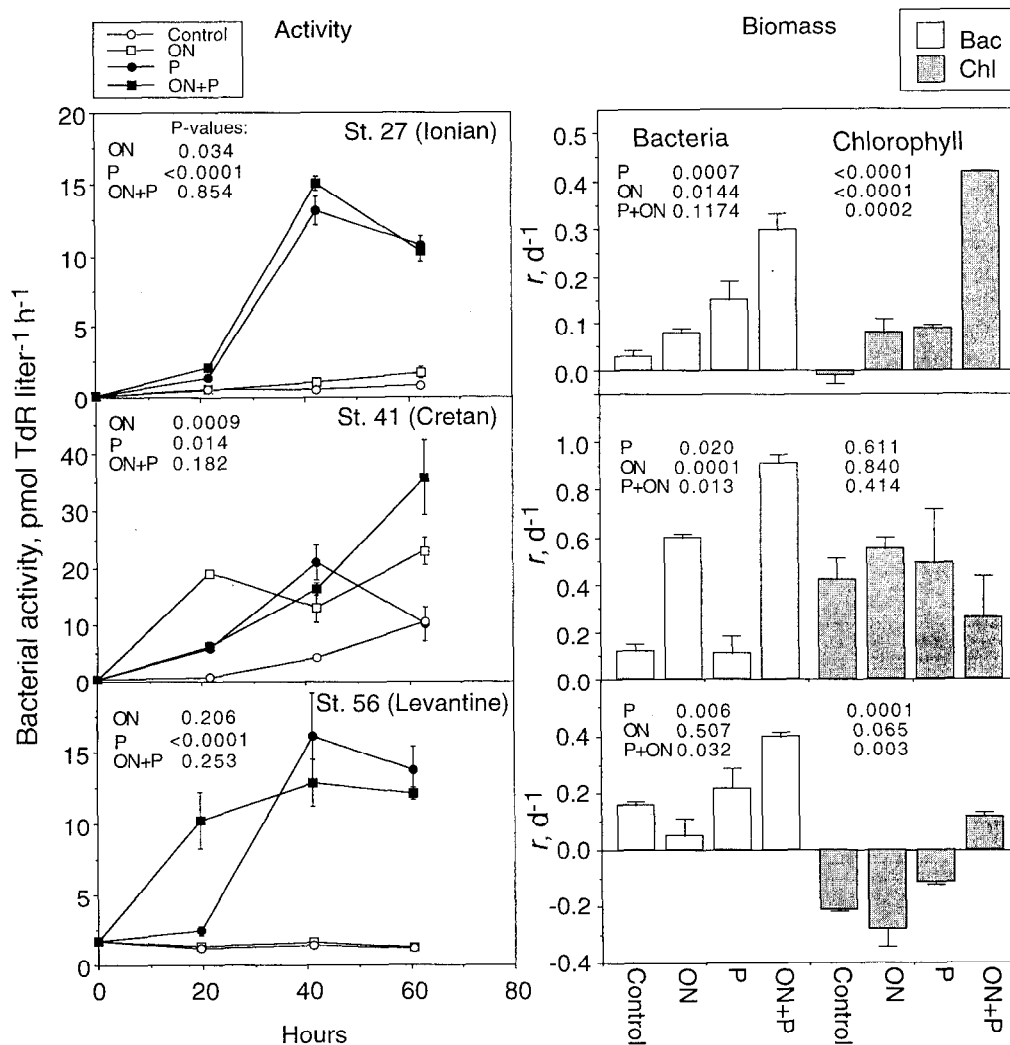


Fig. 3. Results of nutrient-enrichment bioassay experiments (see text for treatment details) using water from the Ionian, Cretan, and Levantine. All data shown are means for three replicate experimental bottles; bars are standard errors. The temporal dynamics of bacterial activity (thymidine method) are shown on the left; the rates of change, r , for bacterial numbers and Chl a are on the right. Results of repeated-measures two-way ANOVA (P values, level of significance) for bacterial activity and of two-way ANOVA for the r values for bacterial numbers and chlorophyll are shown within the figures.

creasing depth bacterial abundance increased relative to that of Chl a .

The rates of bacterial incorporation of thymidine and APA exhibited a matching pattern (Fig. 2). Compared to other stations, bacterial activity per cell and specific APA in the upper 75 m were relatively low. Phosphorus turnover times were distinctly fast in the upper water (3.6 h at 20 m, 6.1 h at 75 m). Notably, the $<1\text{-}\mu\text{m}$ fraction accounted for 77% of PO_4^{3-} uptake in those shallow waters. With increasing depth, PO_4^{3-} as well as bioavailable P concentrations increased (although at 110 m bioavailable P was 8.9 nM, which was unexpectedly low). Similarly, P turnover times increased to >100 h, indicating P sufficiency, and the contribution of the bacterial fraction to ^{32}P uptake declined below 50%, similar to the P-sufficient situation in the Rhodes gyre.

Nutrient addition bioassays—In the nutrient addition bioassays (Fig. 3), bacterial activity was enhanced by P addition in all three experiments, whereas addition of ON enhanced this activity only in the Cretan and Ionian. The magnitude of this enhancement was markedly different for the two additions. After 40–60 h, activity was generally higher by an order of magnitude with P addition compared to no P addition, whereas the enhancement due to ON addition was only slight in the Ionian experiment. In the Cretan experiment, where initial P_i concentrations were higher than in the other experiments (Table 2), the magnitude of the P effect was smaller, while that of ON was larger. Interestingly, a considerable increase in bacterial activity was recorded in the Ionian experiment, even with no nutrient addition in the controls.

A similar picture emerged after bacterial activity was nor-

Table 3. Specific bacterial activity measured with [³H]thymidine (TdR), in pmol TdR h⁻¹ (10⁹ cells)⁻¹ at the start and end of the three nutrient-enrichment experiments (treatments: control, no nutrient addition; ON, addition of other nutrients, i.e. NH₄⁺, Fe, and EDTA; P, addition of orthophosphate; ON+P, addition of all of the above).

Time	Treatment	Sta. 27, Ionian	Sta. 41, Cretan	Sta. 56, Levan- tine
Start	All treatments	0.3	2.7	4.7
End	Control	2.5	49.0	2.2
End	ON	4.7	30.3	3.1
End	P	23.7	47.2	21.4
End	ON+P	15.2	20.9	12.4

malized per bacterial cell (Table 3). In the Ionian and Levantine, considerably higher specific activities were recorded with P addition vs. a much smaller effect for ON addition or no addition. In the Cretan, all four treatments including the control showed a response, which varied from an 8- to 18-fold increase in activity. In fact, the biggest increase was recorded in the control treatment.

The static response measures (bacterial numbers and Chl *a* concentrations) are presented as the rate of change in biomass per day, with positive values indicating net increase and negative values indicating net decline. Thus, these data provide a measure of the net changes in community biomass, as opposed to the bacterial activity parameter, which showed changes at a physiological level. The rate of change in bacterial numbers and Chl *a* revealed similar patterns to those of bacterial activity in that both were significantly enhanced by the addition of P in all experiments, except for chlorophyll in the Cretan. Here, the average rate of change in Chl *a* ranged from 0.26 to 0.55 d⁻¹ in all treatments, including the control. Addition of ON enhanced bacterial biomass in the Ionian and Cretan, and Chl *a* concentrations only in the Ionian.

Unlike the response of bacterial activity in which the two nutrient additions elicited independent responses, interactions between the two nutrient additions were observed for bacterial biomass in the Cretan and Levantine, as well as for Chl *a* in the Ionian and Levantine.

Discussion

We have attempted to determine whether the growth of current phytoplankton and bacterial populations in the eastern Mediterranean was P limited. This was not simple since there is no single method to conclusively demonstrate nutrient limitation. Furthermore, the application of techniques developed for use in more nutrient-enriched waters may not be directly applicable to the extremely low nutrient concentrations and low plankton biomass waters of the area. In these waters there may be a delicate balance between the abiotic and biotic components of the system that cannot be assessed with the existing methods.

To obtain an assessment close to real time, we measured APA, ³²P uptake and turnover time, and the concentrations

of ambient P_i and bioavailable P. Generally, measurements suggested that P was in short supply, potentially limiting microbial growth rates, or at least the biomass attained. Phosphate concentrations in the upper mixed layer of vast areas of the eastern Mediterranean were consistently <40 nM, close to the detection limit of conventional methods. By using radiolabel techniques, we found that the upper limit to bioavailable P (both organic and inorganic) ranged from 6 to 18 nM. Measurements of concentrations of bioavailable P in the Mediterranean have rarely been conducted. The only reference we found was for the unpublished data of Thingstad, Zweifel, and Rassoulzadegan as cited in Vaultot et al. (1996), who quoted a somewhat wider range, 1–30 nM, depending on the methods and assumptions. Such low biologically available P concentrations, about an order of magnitude lower than the half-saturation constant for P uptake (*K_i*) for most phytoplankton species (Cembella et al. 1984), suggest severe P limitation. These low values also agree with those known to limit *Prochlorococcus* growth rate (30 nM; Parpais et al. 1996).

By use of our proposed cutoff of 7 h, the ³²P *T_i* data (Table 2) suggest rapid cycling of P throughout the Ionian and Levantine basins, but not in P_i-enriched sites (Rhodes core, Cretan front, deep water), where *T_i* exceeded 100 h. Similar turnover times (1.6–7 h) were recorded during autumn in Villefranche Bay, northwestern Mediterranean (Dolan et al. 1995), where a considerably higher value (58 h) was recorded after winter mixing.

It has been postulated that under P-limited conditions in oligotrophic systems, bacteria are superior competitors for P uptake, whereas phytoplankton can take advantage of sudden pulses of nutrients (e.g. Suttle et al. 1990). In our study, bacterial-size particles (<1 μm) dominated the cycling of P in the upper mixed layer of P-deficient areas of the eastern Mediterranean, accounting for 60–85% of P uptake (Table 2, Fig. 2). The situation was reversed in P-enriched sites such as waters at depths >100 m and at the Rhodes gyre, where the phytoplankton fraction (>1 μm) accounted for 52–60% of the uptake. Similar to our findings, bacterial dominance of P uptake at low ambient P_i and phytoplankton dominance at high ambient P_i, or when a pulse of P was added to water samples, were reported for the Sargasso Sea (Suttle et al. 1990), at Sandsfjord, Norway (Thingstad et al. 1993), as well as in various freshwater systems (e.g. Suttle et al. 1988; Cotner and Wetzel 1992).

Our APA data were difficult to interpret without DOP and particulate organic P data. Ammerman et al. (1994) and Thingstad and Rassoulzadegan (1995) suggested that in oligotrophic oceans, DOP may be 5–10-fold more abundant than P_i. This could be a possible explanation to our inconclusive APA data. However, our measurements of the upper limit of bioavailable P (sum of bioavailable DOP + DIP) show that in our samples, DOP concentrations could not be much greater than ambient PO₄³⁻ concentrations. Recently, G. Citivarese (pers. comm.) found DOP concentrations from the Adriatic straits and the Ionian Sea to be similar to ambient PO₄³⁻ concentrations of ~60 nM.

Comparison of our specific APA values (nmol MU (μg Chl)⁻¹ d⁻¹) with those available from various eutrophic lakes (e.g. Healey and Hendzel 1980; Pettersson 1980; Chrost and

Overbeck 1987; Newman et al. 1994; Waiser and Roberts 1995) revealed that the rates in the eastern Mediterranean were generally very low, tentatively implying P sufficiency. However, we found no comparative data from oligotrophic oceans. We speculate that, as was the case with ^{32}P turnover times, specific APA values from the ultra-oligotrophic eastern Mediterranean cannot be interpreted on the basis of values from more eutrophic freshwater systems. The low values we found should be reevaluated only after more data from similarly oligotrophic marine systems become available.

As a complementary approach to the real-time measurements, we carried out nutrient-enrichment bioassays. These were conducted while taking into consideration that any manipulation of a natural system is likely to cause changes to the natural microbial communities. The enclosure of these waters in bottle bioassays changes the hydrodynamics (mixing and light), nutrient dynamics, and food-chain structure (grazers). Therefore, these experiments could be used only to test for the potentially limiting nutrient.

In general, P addition in these experiments enhanced bacterial activity and numbers, as well as Chl *a* concentrations. Differences in the magnitude of the P effect depended on the water source and appeared to be related to ambient PO_4^{3-} concentrations. The responses for the PO_4^{3-} -depleted waters (20–40 nM) from the Ionian and Levantine were similar, with strong P effects and small or no effects for other nutrients. In the Cretan, where ambient PO_4^{3-} concentrations were much higher (90 nM), the P effects were small or non-existent. Here, growth was observed even with no nutrient addition, suggesting strongly that these populations may have been limited in situ by other factors such as light or grazing.

Overall, the enrichment bioassays indicated that given physical conditions in the sea that would bring about nutrient limitation such as a strongly stratified and well-lighted mixed layer or a decrease in grazing pressure that allowed increased biomass to exhaust the nutrient supply rates, P would be the most likely nutrient to become limiting to the plankton. There were also indications, however, that in some cases other factors limited the current populations. The growth in the controls of the Cretan Sea bioassay indicated some residual nutrients for plankton growth.

The ultimate test for P limitation of the Mediterranean should be at the ocean level, something similar to the iron-fertilization experiment (Kolber et al. 1994) used to demonstrate Fe limitation of the equatorial Pacific. The convective chimney and upwelling event in the Rhodes gyre naturally provided the nutrient fertilization of otherwise P-limited eastern Mediterranean surface water. The consequences of the ~5-fold increase in euphotic zone ambient P_i concentrations were notable: Chl *a* concentrations in the Rhodes core were double those at other stations, the ratio of bacterial to algal biomass decreased; P uptake was phytoplankton-dominated as opposed to bacterial-dominated elsewhere; and the turnover time of P was thousands of hours vs. 7 h at other stations. Additionally, A. Yilmaz (pers. comm.) found in the Rhodes core during winter 1995 that sestonic N:P ratios were considerably below the Redfield ratio, which she interpreted as being due to luxury uptake of P at a time when P was abundant, in comparison with

ratios that were close to the Redfield ratio in most of the eastern Mediterranean. Interestingly, bacterial numbers in the Rhodes core did not increase above average, suggesting another limitation, possibly labile DOC or grazing. The obvious shortcoming of this natural experiment was that owing to the vertical mixing surface water, nitrate concentrations in the Rhodes gyre were also higher than elsewhere, so that the effect of P enrichment could not be separated from the effect of N enrichment. Furthermore, the deep mixing altered the underwater light climate that the phytoplankton were subjected to, and most likely brought about, light limitation.

Howarth (1988) has noted that it would not be surprising that phytoplankton species in oligotrophic marine waters are growing near their maximum rates since they are obviously well adapted to grow under low-nutrient conditions. Furthermore, he surmised that if the rate of nutrient supply were to be increased, then these species would be replaced by others that are better competitors in the new situation and would have a higher production rate. Relatively little is known about bacterial species composition in marine waters, but it is not unrealistic to assume that Howarth's comments are applicable to bacterial populations. It is therefore possible for phytoplankton and bacteria to be growing near their maximum rates and not be nutrient limited at all (*see* references in Howarth 1988).

On the basis of our data, we cannot rule out that this was the situation during winter in the eastern Mediterranean. As Dolan et al. (1995) have clearly stated, the fact that P turns over rapidly in the Mediterranean Sea does not prove that the microbes can not grow at their maximum rates due to P limitation. Grazing control of biomass is a strong possibility, and at deeply mixing stations (e.g. Sta. 39, 41, 63) it is possible that light limitation was significant even in the very clear waters of the eastern Mediterranean.

Thus, it was evident that in the "oases" where P was relatively more abundant than in most of the eastern Mediterranean "desert," such as the cyclonic Rhodes gyre during upwelling and in a frontal zone in the Cretan Sea, other factors limited microbial biomass and growth. In the ultra-oligotrophic desert waters, a delicate and dynamic balance probably differentiates between times when the microbial populations are nutrient limited and other times when light or grazers are the main limiting factor. The interpretation of results obtained using conventional methods, developed and tested in more enriched systems, may not be valid.

Our study was conducted in January, during winter mixing of the ocean surface waters when surface-water nutrient concentrations are relatively high. In summer and fall these concentrations are typically lower, suggesting that nutrient limitation would be even more severe than observed in this study. Further experimental evidence is still required to demonstrate that P (rather than other nutrients) is potentially the major limiting nutrient at other times of the year.

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